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(54) Use of a feed additive for preventing weight loss, reduction in weight gain, and anorexia due to immune stimulation

Verwendung eines Futterzusatzes zur Vermeidung des durch Immunstimulation verursachten Gewichtsverlustes und Anorexia

Emploi d'un additif alimentaire pour éviter la perte de poids, la réduction de la croissance du poids, et l'anorexie, liées à la stimulation immunitaire

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## Description

The present application generally relates to the use of a composition, e.g. a feed, food or a pharmacologic preparation containing conjugated linoleic acid, free linoleic acid, salts thereof and mixtures thereof.

5 Researchers have observed anorexia and weight loss or reduction in weight gain in humans and animals that have been exposed to immune stimulants, such as endotoxin (LPS). The intraperitoneal injection of lipopolysaccharide (i.e. endotoxin) into chickens decreases food intake and growth rate for 24 hours, alters nutrient metabolism, and induces fever.

Recent studies (Klasing et al., 1987, J Nutr. 117:1629) have confirmed that the vaccination of domestic fowl with 10 several immune stimulants also can result in a substantial reduction in feed intake and induce weight loss or decreases in weight gain. In a study recently conducted with white Pekin ducks, two vaccinations reduced final carcass weight by as much as 0.4 lbs./bird and breast meat by 0.075 lbs./bird. Broilers and Single Comb White Leghorns (egg laying chickens) also have been observed to have reduced weight gains following immune stimulation. The potential losses due to immune stimulation costs the poultry industry millions of dollars per year. At the present time, antibiotics are used 15 to prevent such weight loss but the use of antibiotics for this purpose is expensive and not without disadvantages.

In a similar manner anorexia, weight loss, and reduced growth of humans that are subjected to chronic immune stimulation because of infections, surgery, or exposure to immune stimulants is devastating to health and well being.

The mechanism by which immune stimulation causes anorexia, weight loss and reduced growth is known to be mediated by catabolic hormones released following immune stimulation (i.e., macrophage cytokine known as interleukin- 1 or IL-1). The production of IL-1 from macrophages simultaneously stimulates T-cells to release IL-2, an anticarcinogenic 20 compound which is desirable, but the release of IL-1 and other catabolic hormones from stimulated macrophages and possibly other immunoregulated cells induces an undesirable systemic reduction in skeletal muscle synthesis and increased muscle degradation resulting in weight loss or a decline in weight gain. Thus, while IL-1 and related immune hormones are essential cytokines for immune function, their systemic hormonal effects are devastating and have prevented its acceptance for immune therapy.

25 There is a need for feed additives, pharmacologic agents, and methods which can enhance growth and prevent the weight loss and anorexia that follows immune stimulation.

It is an object of the present invention to provide for this need and to avoid the disadvantages mentioned above.

This problem is solved by using an effective amount of a member selected from conjugated linoleic acid, free linoleic 30 acid, salts thereof and mixtures thereof for the manufacture of a composition for preventing weight loss, reduction in weight gain or anorexia in an animal or human caused by immune stimulation. Preferred embodiments are subject-matter of claims 2 to 7.

The invention is due to the realization that especially the conjugated linoleic acids 9,11-octadecadienoic acid and 10,12-octadecadienoic acid (CLA) are valuable feed or food additives and potential pharmacologic agents which can 35 enhance growth and prevent the weight loss that follows immune stimulation in animals, including humans.

It will be apparent to those skilled in the art that the forementioned objects may be achieved by the practice of the present invention.

In a preferred embodiment of the invention a safe and effective amount of CLA is added to the food of an animal or human which has been or may be subjected to vaccination or other exposure to immune stimulants. The amount of CLA 40 to be used as a feed or food additive is an amount which is safe and effective under conditions of use to prevent weight loss and/or enhance the growth of the animal to which it is administered. Because of the difference in size and susceptibility of animals and humans to the adverse effects of immune stimulation the amounts which are safe and effective will vary considerably. However, since CLA is a natural food ingredient and it is relatively non-toxic, the amount which can be administered is not critical as long as it is enough to be effective.

45 The practice of the present invention is further illustrated by the examples which follow:

## Example 1

Four pens of 10 chicks were fed a standard poultry ration with 0.5% lard (controls) or with 0.5% CLA mixed daily (2 50 pens per treatment). When the chicks were 3 weeks of age, they were weighed, inoculated with 100µg of E. Coli 0111:B4 endotoxin i.p. to stimulate the immune system. Chicks were again weighed 24h later. While the chicks fed the unsupplemented diet failed to gain body weight following endotoxin exposure, the chicks fed CLA gained 10 grams (p<.07)

(Table I). Antibody responses to sheep red blood cells demonstrated that CLA had no effect on antibody synthesis.

Table I

Treatment	Av. Initial Wt.	Av. Wt. 24h post endotoxin	Av. initial 24h	% with no or negative gain
Control	311 $\pm$ 12	311 $\pm$ 12	0 $\pm$ 3	53
.5% CLA	305 $\pm$ 9	315 $\pm$ 9	10 $\pm$ 4	27

#### Example 2

Another group of chicks were fed a diet containing 0.5% CLA which was mixed with the feed daily. At 3 weeks of age the chicks were inoculated i.p. with 750 $\mu$ g E. Coli 055:B5 endotoxin to stimulate immunity or phosphate buffered saline (PBS) as a control. The control chicks injected with PBS gained 9 g over the following 24h period, and the CLA fed, PBS injected chicks gained 13.5 g. When chicks fed the control diet were injected with endotoxin, they lost 1.3 g of body weight over the following 24h period. However, the CLA fed chicks even after endotoxin injection continued to gain an average of 6.6 g.

The results of the examples demonstrate that a lower proportion of chicks lose weight, within 24 hours of being injected with endotoxin, when the chicks ingest an animal feed which contains CLA. In fact, the results show that not only do a fewer number of birds lose weight but that those birds that are fed CLA actually gain considerably more weight than the control birds. In addition, the loss of body weight in rats following stimulation was 50% of those not fed CLA.

#### Example 3

A group of seven rats was fed a semi-purified diet to which CLA was not added; a second group was fed the same diet containing 0.5% CLA. Three weeks later the animals were weighed. Four animals from each group were inoculated with endotoxin (1 mg/kg body weight); the remaining three animals from each group were inoculated with PBS. Rats fed the control diet and injected with PBS gained 7.4 g. Rats fed the CLA-containing diet and injected with PBS gained 5.4 g. Rats fed control diet and injected with endotoxin lost 21.05 g. Rats fed CLA containing diet and injected with endotoxin lost only 11.9 g.

In another embodiment of the invention a fatty acid that is converted into CLA or which modulates the level of CLA in the body of an animal or a human is fed. Specifically, we have found that linoleic acid is converted to CLA in the bodies of rats, probably by microorganisms in the gastrointestinal system (S.F. Chin, W. Liu, K. Albright, and M.W. Pariza, 1992, FASEB J. 6:Abstract #2665).

#### Example 4

A group of seven rats was fed a semi-purified diet containing 5% corn oil; a second group was fed the same diet with corn oil but also containing added free linoleic acid (0.5%). Three weeks later the animals were weighed. Four animals from each group were inoculated with endotoxin (1 mg/kg body weight); the remaining three animals from each group were inoculated with PBS. Rats fed the control diet and injected with PBS gained 7.4 g. Rats fed the diet to which linoleic acid had been added, and injected with PBS, gained 7.2 g. Rats fed control diet and injected with endotoxin lost 21.05 g. Rats fed diet to which linoleic acid had been added, and injected with endotoxin, lost only 11.4 g. We believe these results are due to the conversion of added linoleic acid to CLA within the body of rats as discussed above.

The methods of the present invention may take several embodiments. In one embodiment, the CLA is added to an animal's feed or to a human's food. In another embodiment, the CLA can be administered to an animal in a pharmaceutical or veterinary composition containing a safe and effective dose of the CLA. In a third embodiment, the animal can be fed a safe amount of the reactants which will form the CLA *in situ* in the animal or human.

The novel animal feeds and pharmaceutical preparations of the present invention are those containing the free conjugated linoleic acids (CLA) 9,11-octadecadienoic acid and 10,12-octadecadienoic acid in combination with a conventional animal feed (e.g. poultry feed), human food supplement, or approved pharmaceutical diluent. Active forms of CLA also include compositions, containing the active isomers of CLA; non-toxic salts thereof; and mixtures thereof. Animals and humans may also be given a substance such as linoleic acid which is converted to CLA within the body, or which may modulate intracellular levels of CLA or otherwise mimic the beneficial effects of CLA in mitigating anorexia, weight loss, and reduced growth resulting from immune stimulation.

The free conjugated linoleic acids (CLA) have been previously isolated from fried meats and described as anticarcinogens by Y. L. Ha, N. K. Grimm and M. W. Pariza, in Carcinogenesis Vol. 8, No. 12, pp. 1881-1887 (1987). Since

then, they have been found in some processed cheese products. Y.L. Ha, N. K. Grimm and M. W. Pariza, in J. Agric. Food Chem., Vol. 37, No. 1, pp. 75-81 (1987). However, the use of animal feeds containing CLA, or its non-toxic derivatives, such as the sodium and potassium salts, in combination with conventional animal feeds or human foods according to the invention is novel.

The free acid forms of the CLA may be prepared by isomerizing linoleic acid. The non-toxic salts of the free CLA acids may be made by reacting the free acids with a non-toxic base. Natural CLA may also be prepared from linoleic acid by the action of  $\Delta^{12}$ -cis,  $\Delta^{11}$ -trans isomerase from a harmless microorganism such as the Rumen bacterium *Butyrivibrio fibrisolvens*. Harmless microorganisms in the intestinal tracts of rats and other monogastric animals may also convert linoleic acid to CLA (S.F. Chin, W. Liu, K. Albright and M.W. Pariza, 1992, FASEB J.6:Abstract #2665).

The CLA obtained by the practice of the described methods of preparation contains one or more of the 9,11-octadecadienoic acids and/or 10,12-octadecadienoic acids and active isomers thereof. It may be free or bound chemically through ester linkages. The CLA is heat stable and can be used as is, or dried and powdered. The CLA is readily converted into a non-toxic salt, such as the sodium or potassium salt, by reacting chemically equivalent amounts of the free acid with an alkali hydroxide at a pH of about 8 to 9.

Theoretically, 8 possible geometric isomers of 9,11- and 10,12-octadecadienoic acid (c9,c11; c9,t11; t9,c11; t9,t11; c10,c12; c10,t12; t10,c12 and t10,t12) would form from the isomerization of c9,c12-octadecadienoic acid. As a result of the isomerization, only four isomers (c9,c11; c9,t11; t10, c12; and c10,c12) would be expected. However, of the four isomers, c9,t11- and t10,c12-isomers are predominantly produced during the autooxidation or alkali-isomerization of c9,c12-linoleic acid due to the co-planar characteristics of 5 carbon atoms around a conjugated double-bond and spatial conflict of the resonance radical. The remaining two c,c-isomers are minor contributors.

The relatively higher distribution of the t,t-isomers of 9,11- or 10,12-octadecadienoic acid apparently results from the further stabilization of c9,t11- or t10,c12-geometric isomers, which is thermodynamically preferred, during an extended processing time or long aging period. Additionally the t,t-isomer of 9,11- or 10,12-octadecadienoic acid that was predominantly formed during the isomerization of linoleic acid geometrical isomers (t9,t12-, c9,t12- and t9,c12-octadecadienoic acid) may influence the final ratio of the isomers or the final CLA content in the samples.

Linoleic acid geometrical isomers also influence the distribution of minor contributors (c,c-isomers of 9,11- and 10,12-, t9,c11- and c11,t12-octadecadienoic acids). The 11,13-isomer might be produced as a minor product from c9,c12-octadecadienoic acid or from its isomeric forms during processing.

To enhance growth, prevent weight loss, the CLA and its non-toxic derivatives, such as the non-toxic salts, in addition to being added to an animal's feed or human food or formed *in situ* can be administered in the form of pharmaceutical or veterinary compositions, such as tablets, capsules, solutions or emulsions to the animal or the humans. The exact amount to be administered, of course, depends upon the form of CLA employed, the route of administration, and the nature of the animal's or human's condition or disease. Generally, the amount employed of CLA and its non-toxic salts employed as a pharmaceutical will range from about one part per million (ppm) to about 10,000 ppm of CLA in the animal's or human's diet. However, the upper limit of the amount to be employed is not critical because CLA is relatively non-toxic and it is a normal constituent of the human diet (including human breast milk). The amounts to be added to a conventional animal feed or human's food as an additive can range from 0.01% to 2.0% or more by weight of the animal's or human's food.

The preferred pharmaceutical and veterinary compositions of CLA contain the non-toxic sodium or potassium salt of CLA in combination with a pharmaceutical diluent. When the compositions are solutions or suspensions intended for oral administration the diluent will be one or more diluents, such as lactose or starch, and the product will be a tablet, capsule or liquid. When the compositions are solutions or suspensions intended for parenteral administration the preferred diluent will be Sterile Water for Injection U.S.P.

#### 45 Claims

1. Use of an effective amount of a member selected from conjugated linoleic acid, free linoleic acid, salts thereof and mixtures thereof for the manufacture of a composition for preventing weight loss, reduction in weight gain or anorexia in an animal or human caused by immune stimulation.
2. Use according to claim 1 in which the composition is a feed or food.
3. Use according to claim 2 in which the effective amount ranges from 0.01 % to 2.0 % by weight of the feed or food.
4. Use according to claim 1 in which the composition is a pharmaceutical or veterinary composition.
5. Use according to anyone of the preceding claims in which the animal is a bird.

6. Use according to anyone of the preceding claims in which the immune stimulation is caused by the inoculation with endotoxin.

5 7. Use according to anyone of the preceding claims in which the conjugated linoleic acid is selected from 9,11-octadecadienoic acid and 10,12-octadecadienoic acid.

#### Patentansprüche

- 10 1. Verwendung einer wirksamen Menge eines Vertreters, gewählt aus konjugierter Linolsäure, freier Linolsäure, Salzen davon sowie Gemischen davon, zur Herstellung einer Zusammensetzung zur Vermeidung eines Gewichtsverlusts, einer Verringerung der Gewichtszunahme oder von Anorexia, die durch Immunstimulation verursacht wurde, bei Tieren oder Menschen.
- 15 2. Verwendung nach Anspruch 1, wobei die Zusammensetzung ein Futtermittel oder ein Nahrungsmittel ist.
3. Verwendung nach Anspruch 2, wobei die wirksame Menge im Bereich von 0,01 bis 2,0 Gew.-% des Futtermittels oder des Nahrungsmittels liegt.
- 20 4. Verwendung nach Anspruch 1, wobei die Zusammensetzung ein Arzneimittel oder ein Veterinärmedikament ist.
5. Verwendung nach einem der vorstehenden Ansprüche, wobei das Tier ein Vogel ist.
6. Verwendung nach einem der vorstehenden Ansprüche, wobei die Immunstimulation durch die Impfung mit Endotoxin verursacht wurde.
- 25 7. Verwendung nach einem der vorstehenden Ansprüche, wobei die konjugierte Linolsäure gewählt wird aus 9,11-Oktadecadiensäure und 10,12-Oktadecadiensäure.

#### Revendications

- 30 1. Utilisation d'une quantité efficace d'un membre choisi parmi l'acide linoléique conjugué, l'acide linoléique libre, leurs sels et leurs mélanges pour la fabrication d'une composition pour prévenir la perte de poids, la réduction de la croissance du poids ou l'anorexie chez un animal ou un homme, provoquées par une stimulation immunitaire.
- 35 2. Utilisation selon la revendication 1 dans laquelle la composition est un aliment ou une nourriture.
3. Utilisation selon la revendication 2 dans laquelle la quantité efficace varie de 0,01% à 2,0% par rapport au poids de l'aliment ou de la nourriture.
- 40 4. Utilisation selon la revendication 1 dans laquelle la composition est une composition pharmaceutique ou vétérinaire.
5. Utilisation selon l'une quelconque des revendications précédentes dans laquelle l'animal est un oiseau.
- 45 6. Utilisation selon l'une quelconque des revendications précédentes dans laquelle la stimulation immunitaire est provoquée par l'inoculation avec une endotoxine.
7. Utilisation selon l'une quelconque des revendications précédentes dans laquelle l'acide linoléique conjugué est choisi parmi l'acide 9,11-octadécadiénoïque et l'acide 10,12-octadécadiénoïque.

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